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Full Length Research Paper

Morphoagronomic and productive traits of RR® soybean due to inoculation via *Azospirillum brasilense* groove

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In the last decades, the Brazilian soy productive chain has passed through a transformation process in which the yield, efficiency, profitability, economic and environmental sustainability are very important issues. In this context, the introduction of microorganisms has provided an increase in grains yield. The objective of this study was to evaluate the inoculation of *Azospirillum brasilense* associated with *Bradyrhizobium japonicum* in agronomic traits and the soybean productivity. The experiment was conducted in randomized blocks in 4 × 2 factorial, with four cultivars (Anta 82 RR®, BRS Favorita RR®, BRS 780 RR®, BRS 820 RR®) and two treatments with *A. brasilense* (inoculated and non-inoculated) with three replications in two growing seasons. The following traits were evaluated: plant height, shoot dry biomass, chlorophyll content, leaf nitrogen content at flowering; and at harvest, the plant height, the insertion of the first pod, number of pods per plant, number of grains per pod, thousand-grain weight , grain yield, and grain harvest index were evaluated. There was a significant effect of growing seasons and cultivars in an isolated way and their interaction in most traits. The conditions in which the study was conducted, with or without inoculation of *A. brasilense* associated with *B. japonicum* do not affect the agronomic traits and grain yield in RR® soybean cultivars.

Key words: *Glycine Max* (L.) Merrill, growth promoting bacteria, rhyzobium.

INTRODUCTION

Soybean [*Glycine max* L. Merrill] is an oilseed of great economic importance in the national and international market due to the high levels of protein and oil in their seeds/grains (Lima et al., 2015). The cultivation has been widely studied due to its high nutritional value and great consumer market. FAO (2013) reported that the soybean produced is enough to provide one-third of the global need of food proteins, since it is completely intended for

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Table 1. Chemical composition of a typical oxisol (0.0-0.2 m) before the experiment installation, Lavras-MG, Brazil, for the 2013/2014 and 2014/2015 growing seasons.

Growing season	pH	Ca^{2+}	Mg^{2+}	Al^{3+}	$H^{+}+Al^{3+}$	SB	CEC	P	K	MO	V
	H_2O	$cmol_c\ dm^{-3}$						$mg\ dm^{-3}$		$dag\ kg^{-1}$	%
2013/14	6.4	5.0	1.4	0	2.9	6.7	9.6	11.46	118	3.41	69.82
2014/15	6.2	3.80	0.80	0	0.9	4.8	5.7	20.83	92	2.23	83.52

H + Al: Potential acidity; SB: sum of bases; CEC: cation-exchange capacity at pH 7.0; MO: organic matter; V: base saturation.

human consumption.

In this scenario, Brazil is the second largest world producer and exporter of soybeans, with 31,902,400 ha^{-1} of sown area, with an average grain yield of 3011 kg ha^{-1} in the 2014/2015 harvest (CONAB, 2015). The Brazilian soy productive chain has undergone modernization processes, which provide the increase in grain yield (Zuffo et al., 2015a).

The introduction of *Bradyrhizobium*, which performs the biological nitrogen fixation (BNF), was one of the major drivers for large-scale soy cultivation in Brazil (Zuffo et al., 2015b). Therefore, the technological advances in soil microbiology area are important for the viability of soybean cultivation. Besides these bacteria, the soil is an ecosystem that has a great variety of plant growth promoting bacteria (PGPBs) that can be free-living or associated with plant roots.

For Silveira and Freitas (2007), PGPBs constitute any bacteria that have beneficial effects on the growth of one or more vegetal species, except for the rhizobia that despite the beneficial relation to vegetal growth, is the result of a symbiotic relationship. In the literature, the most studied diazotrophs bacteria as associative PGPBs, belong to the genus *Azospirillum*. Mainly due to its use as inoculants commercialized in Brazil with a recommendation for the grasses, Hungria (2011) while using the *Azospirillum,* noted increases of 31 and 26% for grain yield in wheat and corn crops, respectively, but with supply of part of the nitrogen required by the plant by the mineral fertilizer.

For Araujo (2008), the *Azospirillum* has the following advantages: the bacteria are endophytic, that is, penetrates the roots of plants; presents antagonism to pathogens; produces phytohormones; it is not very sensitive to temperature variations; and occurs in all kinds of soil and climate. Among the plant hormones, research has demonstrated the ability of *Azospirillum brasilense* in producing auxin, gibberellins, and citocianinas under "*in vitro*" conditions (Masciarelli et al., 2013).

The use of inoculation with *A. brasilense* in leguminous plants has been studied; however, the effects are still contradictory. Reports presented by Bárbaro et al. (2009), Hungria et al. (2013) and Hungria et al. (2015) show influences on the agronomic traits of soybean crop, but the results checked by Gitti et al. (2012) and Zuffo et al. (2015a), do not support the authors mentioned earlier.

Therefore, the objective of this study was to evaluate the *A. brasilense* inoculation associated with *Bradyrhizobium japonicum* and its influence on agronomic traits and soybean yield.

MATERIALS AND METHODS

The experiment was conducted in the 2013/2014 and 2014/2015 growing seasons, in Lavras - MG, at the Scientific and Technological Development Center of Agriculture – Muquem Farm, located at latitude 21°12'S, 44°58'W longitude and altitude of 918 m in soil classified as Dystroferric Red Latosol - Oxisol, with clayey texture, with the following textural values: Clay: 640 g kg^{-1}; Silt: 200 g kg^{-1}; Sand: 160 g kg^{-1}. The chemical composition of the experimental area soil is shown in Table 1.

The climate is Cwa according to the Köppen classification, with average annual temperature of 19.3°C and normal annual rainfall of 1,530 mm (Dantas et al., 2007). Climatic data during the experiments were collected at the weather station of the National Institute of Meteorology (INMET) located at the Federal University of Lavras-UFLA and are presented in Figure 1.

The experimental design was a randomized block, arranged in a 4 × 2 factorial, with four cultivars (Anta 82 RR®, BRS Favorita RR®, BRS 780 RR®, BRS 820 RR®) and two treatments with *A. brasilense* (inoculated and non-inoculated) with three replications. Each plot consisted of four sowing lines of 5 m in length spaced in 0.50 m, and the area of each plot was of 10 m^2 (5 m × 2 m). The two central rows were considered as the useful area.

The sowing was carried in November of each growing season. Fertilization consisted of 350 kg ha^{-1} of the N-P_2O_5-K_2O (02-30-20) formulated, applied via groove. *B. japonicum* (Brad) and *A. brasilense* (Azos) bacteria were inoculated via groove after soybean sowing. The dosage of *B. japonicum* was 18 ml kg^{-1} of seed - SEMIA 5079 and 5080 strains, containing 10.8×10^6 CFU/seed of the inoculant Nitragin Cell Tech HC® (3×10^9 CFU/ml). *A. brasilense* used the dosage of 6 ml kg^{-1} of seed - AbV5 AbV6 strains, containing 24×10^4 CFU/seed of the inoculant Azo® (1×10^8 CFU/ml).

The microorganisms inoculation was carried out using a motorized backpack sprayer, coupled to the bar with four spray tips XR 110.02, applying spray volume equivalent to 150 L ha^{-1}. First, there was inoculated *B. japonicum* and then the *A. brasilense*.

At the beginning of flowering (R_1) plant height with assistance of a millimeter rule, shoot dry biomass using a forced air circulation oven at 60°C for 72 h until constant weight, with posterior plant residues weighting were determined. The collection of leaves (third trifoliate from top to bottom) was also held, then washed in deionized water and placed with the shoot dry biomass for drying. The dried leaves were ground in a Wiley mill. Chemical analysis of leaf tissue of macro and micronutrients were conducted according to the methodology described by Sarruge and Haag (1974); leaf chlorophyll content using chlorophyll portable model SPAD 502 Plus® by measuring 3 points in each trifoliate leaf in different parts

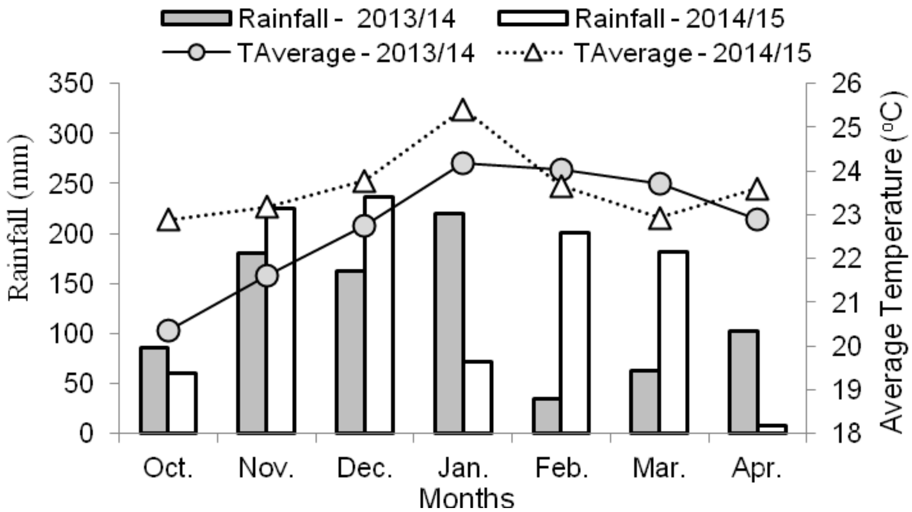

Figure 1. Monthly means for rainfall and air temperature in Lavras-MG, Brazil, for 2013/2014 and 2014/2015 growing seasons, during the experiment evaluations (National Institute of Meteorology (INMET).

on the same leave, always in the leaf blade between the nerves in the third trefoil from top to bottom.

At harvest, the following traits were obtained: plant height and insertion of the first pod with millimeter ruler assistance; then the collection of five plants per plot was held to assess the number of pods per plant and number of grains per pod through manual counting; thousand grain weight according to the methodology described in Brasil (2009); grain yield standardized to grain moisture of 13% in Kg/ha^{-1}. The grain harvest index (GHI) was also determined in the following way: GHI = grain yield/grains productivity + straw.

Analyses of individual and joint variances were performed adopting the statistical model and an analysis procedure similar to that presented by Ramalho et al. (2012). The means were grouped by the Scott-Knott test (1974). Statistical analysis was performed with assistance of statistical package SISVAR® (Ferreira, 2011).

RESULTS AND DISCUSSION

Except for plant height on flowering, nitrogen amount on leaf tissues and height of insertion of the first pod for the growing season were found that to have a significant ($P≤0.01$) influence on the studied variables (Tables 2 and 3). This results are in accordance with Felisberto et al. (2015) who also observed differences on soy agronomic variables according to the crop year.

The number of grain per pod and the grain harvest index were not significantly influencied by cultivars (Table 2). Soares et al. (2015a) and Felisberto et al. (2015) observed an effect of cultivars on the agronomic traits evaluated. The authors affirm that it was expected, since cultivars have different genetic background, growth habit, maturation group and other characteristics providing variations.

Regarding the inoculation with *A. brasilense*, no statistical differences on the evaluated characteristics were observed. Zuffo et al. (2015b) also observed no differences with the inoculation of soybean seeds with only *A. brasilense* or co-inoculated with *B. japonicum* on the plant height, number of trefoil, shoot dry biomass and roots, dry mass of nodules, root volume, chlorophyll content and leaf nitrogen, in accordance with the results of this study.

When the interaction between cultivar × growing season was studied, no statistical differences were observed for the first pod insertion height, yield and production of grain components (Tables 2 and 3). The interaction between cultivar × growing season was also verified by Soares et al. (2015b), indicating that the responses, considering the environmental variations, were not the same for the evaluated cultivars. Since the growing seasons presented differences for rainfall occurrence and temperatures, variations that are unpredictable were expected and the researcher do not have control.

For the interactions between growing season × *A. brasilense*, cultivar × *A. brasilense* and growing season × cultivar × *A.brasilense,* no statistical differences were observed (Tables 2 and 3). Therefore, it can be inferred that the inoculation with *A. brasilense* have no relation with cultivar and growing season. This fact can be explained by the absence of variability of the “variation cause” (*A. brasilense*).

The highest values for shoot dry biomass, chlorophyll content, plant height on harvest, number of pods per plant, number of grains per pod, grain yield and harvest index were obtained during the 2013/2014 growing season. It was due to the climatic conditions (Figure 1), mainly during January flowering, when a high pluviometric index was observed for the 2013/2014 growing season.

The highest values for number of pods per plant and

Table 2. Analysis of variance and means for plant height at flowering (PHF), shoot dry biomass (SDB), chlorophyll content (CLC) and N (NC) in leaves tissues of RR® soy on flowering stage (R_1), obtained with inoculation and non-inoculation of *Azospirillum brasilense* with *Bradyrhizobium japonicum* in RR® soy cultivars, to 2013/14 and 2014/15 growing seasons. Lavras-MG, Brazil.

Cause of variation	DF	ANOVA (QM)[1]			
		PHF	SDB	CLC	NC
		cm		unity	
Block (Bl)	2	5.17	113.31	6.86	5.07
Year (Yr)	1	9.72[ns]	15330.20**	171.25**	10.45[ns]
Cultivar (C)	3	255.90**	166.77*	19.19*	49.88**
Azospirillum (Az)	1	9.01[ns]	121.28[ns]	0.14[ns]	7.36[ns]
An × C	3	36.72[ns]	95.70[ns]	3.48[ns]	19.26[ns]
An × Az	1	1.47[ns]	68.45[ns]	6.35[ns]	20.28[ns]
C × Az	3	45.59[ns]	81.31[ns]	2.03[ns]	5.33[ns]
Yr × C × Az	3	5.04[ns]	112.54[ns]	2.07[ns]	0.38[ns]
Bl × Yr	2	22.55[ns]	336.30**	0.01[ns]	0.66[ns]
Error	28	16.12	36.76	5.50	7.29
Mean	-	53.47	57.88	39.54	43.82
CV (%)	-	7.51	10.47	5.93	5.98
Factors			**Means**		
Growing season²					
2013/2014		53.02[a]	75.76[a]	41.43[a]	43.35[a]
2014/2015		53.92[a]	40.01[b]	37.65[b]	44.29[a]
***Azospirillum*²**					
Presence		53.04[a]	59.47[a]	39.48[a]	44.21[a]
Absence		53.90[a]	56.29[a]	39.60[a]	43.43[a]
Cultivars³					
Anta 82 RR®		49.23[c]	62.75[a]	38.17[b]	44.37[a]
BRS Favorita RR®		50.23[b]	55.01[b]	40.69[a]	44.65[a]
BRS 820 RR®		49.23[c]	54.92[b]	38.76[b]	40.84[b]
BRS 780 RR®		59.15[a]	58.86[a]	40.53[a]	45.43[a]

¹** and *significant for 1 and 5% of probability, respectively, for F test. ns: Non-significant; MS: means square; DF: degree of freedom; CV: coefficient of variation. ²Means followed by the same letter have no difference, according to F test. ³Means followed by the same lowercase in the column are from the same group, according to Scott Knott (1974) test at 5% of probability.

number of grains per pod affected directly grain yield and higher harvest index. On the other hand, the thousand-grain weight was lower for this growing season, presumably by the increase of drain (number of pods and number of grain per pod), with less amount of photoassimilates for each grain.

The efficiency of *A. brasilense* soil inoculation on soy culture can be related to the low competition between the soil microflora and the native bacteria from *Azospirillum* genus (Didonet et al., 2000). The authors also conclude that besides the inoculant quality, the inoculation process is crucial to achieve a higher number of viable bacteria. Therefore, it is possible that a competition with *Bradyrhizobium* or native bacteria prevented the beneficial effect of *A. brasilense* on the development of agronomic traits and the soy grain yield on these study conditions.

Studies that showed benefits in using *A. brasilense* in leguminous crops (Hungria et al., 2013, 2015) did not showed benefits of the leguminous inoculation with *Bradyrhizobium japonicum* and *Azospirillum*, possible because of an increase in the nodulation and N_2 fixation or even due to indirect factors that can be involved. The same authors describe that the resistance to water deficit can be increased and Yadegari et al. (2008) affirm that this is due to the involved bacteria. Otherwise, even with a low rainfall index observed on February and March of 2013/2014 (Figure 1), these benefits were not observed in the present study.

In a general manner, cultivars presented satisfactory

Table 3. Variance analysis and means for plant height at harvest (PHH), insertion of the first pod (IFP), number of pods per plant (NPP), number of grains per pod (NGP), thousand-grain weight (TGW), grain yield (GY) and grain harvest index (GHI) on RR® soy cultivars at maturation (R_8), with inoculation and non-inoculation of *Azospirillum brasilense* with *Bradyrhizobium japonicum* on RR® soy cultivars, to 2013/14 and 2014/15 growing seasons. Lavras-MG, Brazil.

Cause of variation	GL	ANAVA (QM)¹						
		PHH	IFP	NPP	NGP	TGW	GY	GHI
		cm		unity		g	Kg ha^{-1}	-
Bloco (Bl)	2	26.64	0.54	1.37	0.01	39.44	19828.26	0.0028
Year (Yr)	1	3898.80**	0.27^{ns}	3560.40**	0.66**	722.68**	8037206.39**	0.1435**
Cultivar (C)	3	81.48*	34.20**	137.92*	0.07^{ns}	4049.74**	1218618.91**	0.0080^{ns}
Azospirillum (Az)	1	18.00^{ns}	0.12^{ns}	65.80^{ns}	0.16^{ns}	10.87^{ns}	9157.97^{ns}	0.0002^{ns}
Yr × C	3	38.32^{ns}	15.38**	251.19**	0.27*	720.77**	543960.63**	0.0061^{ns}
Yr × Az	1	7.52^{ns}	0.96^{ns}	11.40^{ns}	0.04^{ns}	196.62^{ns}	35154.75^{ns}	0.0009^{ns}
C × Az	3	19.20^{ns}	3.91^{ns}	97.42^{ns}	0.04^{ns}	10.46^{ns}	200718.36^{ns}	0.0040^{ns}
Yr × C X Az	3	22.28^{ns}	7.53^{ns}	52.88^{ns}	0.21^{ns}	50.54^{ns}	258302.26^{ns}	0.0012^{ns}
Bl × Yr	2	6.95^{ns}	0.68^{ns}	18.31^{ns}	0.04^{ns}	360.52**	63261.64^{ns}	0.0006^{ns}
Error	28	19.75	2.63	53.69	0.08	87.90	144445.51	0.0028
Mean	-	70.77	13.55	59.10	2.07	158.04	3583.63	0.50
CV (%)	-	6.28	11.67	12.40	13.77	5.93	10.61	10.95
Factors					**Means**			
Growing season²								
2013/2014		79.78^{a}	13.47^{a}	67.71^{a}	2.19^{a}	154.16^{b}	3993^{a}	0.56^{a}
2014/2015		61.75^{b}	13.62^{a}	50.49^{b}	1.95^{b}	161.92^{a}	3175^{b}	0.45^{b}
***Azospirillum*²**								
Presence		71.38^{a}	13.50^{a}	60.27^{a}	2.01^{a}	157.57^{a}	3597^{a}	0.50^{a}
Absence		70.15^{a}	13.60^{a}	57.93^{a}	2.13^{a}	158.52^{a}	3570^{a}	0.50^{a}
Cultivars³								
Anta 82 RR®		72.35^{a}	11.13^{b}	57.36^{b}	2.06^{a}	133.77^{c}	3532^{b}	0.50^{a}
BRS Favorita RR®		66.88^{b}	13.65^{a}	55.18^{b}	2.08^{a}	177.99^{a}	3229c	0.53^{a}
BRS 820 RR®		72.15^{a}	14.80^{a}	62.13^{a}	2.16^{a}	163.03^{b}	4003^{a}	0.47^{a}
BRS 780 RR®		71.70^{a}	14.61^{a}	61.73^{a}	1.96^{a}	157.37^{b}	3571^{b}	0.50^{a}

¹** and *significant for 1 and 5% of probability, respectively, for F test ns: Non-significant; MS: means square; DF: degree of freedom; CV: coefficient of variation. ²Means followed by the same letter have no difference, according to F test. ³Means followed by the same lowercase in the column are from the same group, according to Scott Knott (1974) test at 5% of probability.

agronomic characteristics (Table 2). On the economic point of view, the grain yield is more important than the other components, and all cultivars presented values above average for the growing season in Minas Gerais state - 2658 kg ha^{-1}, achieved during 2014/2015 growing season (CONAB, 2015). The BRS 820 RR® resulted in the grain yield of 4003 kg ha^{-1}, 50% more than the grain yield for the 2014/2015 growing season. However, it should be noticed that BRS 820 RR® is the latest cultivar (RM 8.2) when compared with the others.

For the interaction between cultivar × growing season, it was observed that the first pod insertion height for the cultivar Anta 82 RR® was lower, mainly during the 2014/2015 growing season (Figure 2A). For the number of pods per plant, BRS 829 RR® presented the higher mean for the 2013/2014 growing season (Figure 2B). During the 2013/2014 growing season, no statistical differences were observed between cultivars, otherwise, when the subsequent growing season were evaluated, it was observed that BRS 820 RR® presented the higher values (Figure 2C). For thousand-grain weight, BRS Favorita RR® presented the highest mean during the two evaluated years, but during the 2014/2015 crop year it was not different from BRS 820 RR® cultivar (Figure 2D). For the grain yield, the cultivars BRS 820 RR® and BRS 780 RR® presented better performance for the 3013/2014

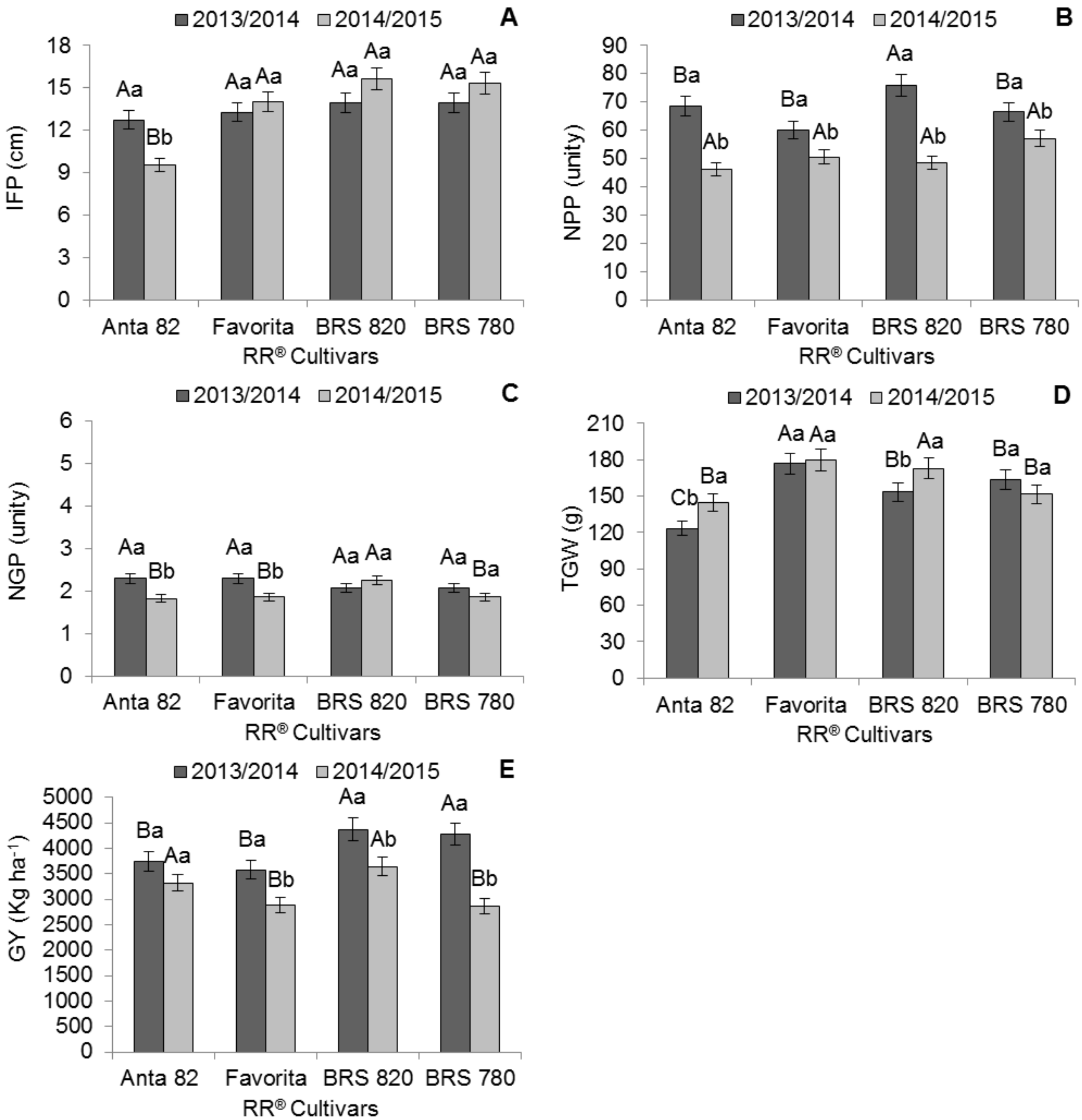

Figure 2. Insertion of the first pod– IFP (A), number of pods per plant – NPP (B), number of grains per pod – NGP (C), thousand-grain weight – TGW (D), grain yield – PG (E) of RR® soy cultivars for the 2013/2014 and 2014/2015 growing seasons, in Lavras, MG, Brazil. Means followed by the same letter, upper case on the same growing season and lower case for the same cultivar, are from the same group, according to Scott Knott (1974) test at 5% of probability.

growing season and the cultivars Anta 82 and BRS 820 RR® during the 2014/2015 growing season, showed the plasticity of the BRS 820 RR® cultivar (Figure 2E).

As previously reported, the interaction between growing season × cultivar is an expected fact and in addition, each cultivar can present intrinsic characteristics and according to the environmental conditions, the agronomic and productive characteristics can be influenced. Thus, characteristics like plasticity of a cultivar are very important, so it can have the ability to modify its morphology depending on environmental conditions.

According to the results and taking into consideration the conditions that this study was conducted, the use or not of *A. brasilense* inoculation, associated with *B. japonicum* did not affect the evaluated agronomic variables and the grain yield in RR® soy cultivars.

Conflict of interests

The authors have not declared any conflict of interests.

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